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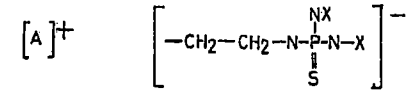
(54) THIOPHOSPHAMIDE DERIVATIVES OF ISOQUINOLINE ALKALOIDS, METHOD OF PRODUCING THEM AND APPLICATION THEREOF

(71) We, LVOVSKY GOSUDARSTVENNY MEDITSINSKY INSTITUT, of 69, Pekarskaya Ulitsa, Lvov, U.S.S.R., a Corporation organised and existing under the Laws of the Union of Soviet Socialist Republic, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to thiophosphamide derivatives of isoquinoline alkaloids and a method for the preparation thereof.

According to the present invention there is provided a compound of the general formula I:—

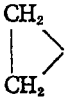


I

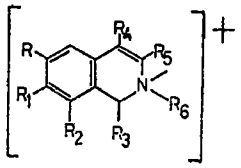
wherein X is the group

A—CH_2—CH_2—

or the group

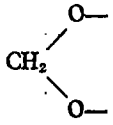


and A is the radical



II

in which each of R, R_1 and R_2 is independently a hydrogen atom or a methoxy group or R and R_1 together or R_1 and R_2 together form a



group; R_3 is a hydrogen atom, a hydroxy group or a methyl group; each of R_4 and R_5 is independently a hydrogen atom or a dihydro-group and R_6 is a hydrogen atom or R_4 and R_6 together or R_5 and R_6 together form part of a fused substituted naphthalene ring.

The said compounds of the above formula are fine-crystalline powders of yellowish or light brown colour; they are readily soluble in dimethyl sulphoxide, dimethyl formamide, chloroform and other organic solvents, and slightly soluble in water.

The new compounds are pharmacologically active and are applicable in medicine as preparations for treating malignant tumors; they also find application as chemical mutagens for the hybridization of agricultural plants and microorganisms thus making them suitable as pesticides and insecticides.

The most active substances of the above specified class of compounds are the N-berberinolethylamide of di-(ethyleneimido) thionophosphoric acid, the tri - (N - sanguinarinol - ethylamide) of thionophosphoric acid, and the thionophosphamide derivatives of the isoquinoline alkaloids extracted from *Chelidonium majus L.*

According to the invention, the N-berberinolethylamide of di-(ethyleneimido) thionophosphoric acid, the tri - (N - sanguinarinol-ethylamide) of thionophosphoric acid and the thionophosphamide derivatives of the isoquinoline alkaloids from *Chelidonium majus L.* are used as active principles in medicinal

preparations for treating malignant tumors. Said preparations can be used for treating tumors of the thoracic gland, ovary, uterine neck, urinary bladder, prostrate, larynx, oesophagus, and other organs. The mechanism of their action resides in the fact that they influence the intimate mechanisms of the cyto-metabolism which are responsible for disturbances in the synthesis of nucleic acids and for greater manifestation of the aerobic respiration phase.

In experiments on rats of strai OR and mice that had experimental tumors different with respect to the histologic structure and origin thereof (Guerin's carcinoma, Walker's carcinosarcoma, Crocker's sarcoma, haematoma PC-1, ascitic carcinoma of ovaries), all of the above-cited preparations cause complete resolving of tumors in the majority of test animals, with the exception of the OR strain rats. At the same time, there are certain differences in the spectrum of the antitumoric effect. Thus, for example, the N-berberinolethylamide of di-(ethyleneimido) thionophosphoric acid and the tri-(N-sanguinarinol-ethylamide) of thionophosphoric acid in doses of adequate toxicity feature a more pronounced effect with respect to the experimental tumors of the liver, which fact is associated with a certain selective action of the alkaloids comprised in said compounds on the hepatic tissue.

A distinctive feature of the antitumotic of the new preparations is the absence of the latent period, which results in their effect being manifest even after one injection, this being of special importance when treating intensively growing tumors and precluding the development of drug resistance of the tumor cells. The manifestation of the antitumoric effect of the new compounds sharply differs from that of the starting compounds, that is, of the isoquinoline alkaloids and thiophosphamide. Thus, for example, the mixture of alkaloids of *Chelidonium majus* causes only 25.7 percent inhibition of the growth of Guerin's carcinoma and 28.5 percent inhibition of the ascitic carcinoma in rats of the OR strain, and thiophosphamide causes 97 and 37 percent inhibition respectively (in case of a sharply pronounced leucopenia). The application of the new preparation causes the inhibition of the same kind of tumors by as much as 98 and 60 percent, respectively.

In contradistinction to the majority of the existing antitumoric preparations, the said new preparations, even in maximum tolerated doses, have no depressive effect on haematopoiesis, and therefore they can be used in combination with other antitumoric preparations, as well as with beam therapy technique. Said new preparations feature a lower toxicity than the initial compounds. The LD_{50} of thiophosphamide is 3—10 mg/kg, that of the alkaloid berberine is 21 mg/kg, and the LD_{50} of the product of their interaction, when administered by intraabdominal injection, is 34.2 mg/kg for mice and 33 mg/kg for rats. The LD_{50} of the product of interaction of thiophosphamide with sanguinarine is 21 mg/kg, that of the thiophosphamide derivatives of the isoquinoline alkaloids of *Chelidonium majus* is 227.1 mg/kg and 302.1 mg/kg.

The action of the preparation, *viz.*, of the thiophosphamide derivatives of the isoquinoline alkaloids of *Chelidonium majus*, was clinically tested in about 300 patients. The preparation was applied for treating tumors of the thoracic gland, ovaries, uterine neck, urinary bladder, prostate, larynx, oesophagus, and other localizations. A noticeable antitumoric effect (resolving of the main tumor and metastatic nodes) was observed in 35—45 percent of the patients with serious forms of the process in the III—IV stage.

The preparation was administered by intramuscular injections in doses increasing from 0.25 to 1 mg/kg, and also in the form of ointments and suppositories.

The course of treatment consisted of 15—20 intramuscular injections with intervals of 48 hours therebetween. After a month break a second course of treatment was prescribed, and then after 3, 6 and 12 months courses of treatment were prescribed for precluding recidivation cases.

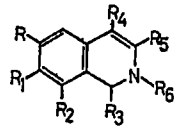
According to the invention, the medicinal preparations comprise an active principle in combination with a pharmaceutical carrier. It is preferable to use a diluent of the following composition (in parts by weight): water, 1.5; polyethylene glycol with the M.W. 400, 1.5; dimethyl sulphoxide, 2. It is expedient that the content of the active principle in the solution should be 0.3—0.5% wt. %.

The medicinal preparation may comprise the active principle in combination with an ointment base. As the ointment base use is made of medicinal petroleum jelly and anhydrous lanoline with the addition of a diluent of the following composition (in parts by weight): water, 1.5; polyethylene glycol with the M.W. 400, 1.5; dimethyl sulphoxide, 2. It is expedient that the content of the active principle in the ointments should be 0.3—0.5 wt. %. It is likewise recommendable to use a medicinal preparation which comprises the active principle in combination with a pharmaceutical base for suppositories. As the base for suppositories use should be made of cacao oil and anhydrous lanoline with the addition of a diluent of the following composition (in parts by weight): water, 1.5; polyethylene glycol, M.W. 400, 1.5; dimethyl sulphoxide, 2. It is preferable to employ suppositories containing 0.03 to 0.05 wt. % of the active principle.

Said medicinal preparations are contra-indicated in such cases as terminal stages of the illness, grave troubles of kidneys with renal insufficient phenomena, grave troubles of the cardiovascular system with cardiac insufficiency phenomena.

Said medicinal preparations do not lose their activity when stored for as long as 2 years under low temperature conditions in premises protected from incident light.

Further according to the invention, the said compounds of the invention are produced by reacting thiophosphamide with an isoquinoline alkaloid of the general formula III:



III

wherein R, R_1, R_2, R_3, R_4, R_5 and R_6 are as defined hereinbefore or with a mixture of alkaloids of the said formula, in a medium of an organic solvent at the boiling point thereof.

To increase the yield of the target product, it is preferable to use benzene, chloroform or dioxane as organic solvents.

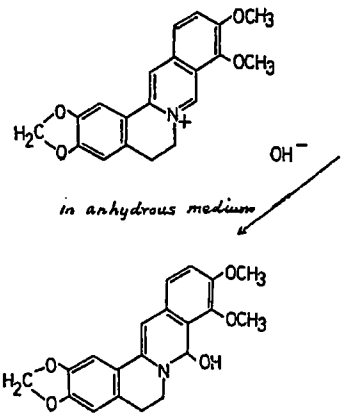
For producing the N - berberinolethylamide of di - (ethyleneimido)thionophosphoric acid, thiophosphamide is reacted with berberine in dioxane at the boiling point of the latter, with a subsequent isolation of the target product.

For producing the tri - (N - sanguinarinol)-ethylamide of thionophosphoric acid, thiophosphamide is reacted with sanguinarine in benzene at the boiling point of the latter, with a subsequent isolation of the target product.

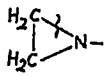
The present method is preferably effected as follows.

The starting compounds, that is, thiophosphamide and the isoquinoline alkaloid, or a mixture thereof, are inter-reacted in the medium of an organic solvent at the boiling point of the latter. The ethylene imine rings of the thiophosphamide are aminolyzed with the formation of the desired products.

Alkaloids of the group of quaternary ammonium bases (such as berberine, sanguinarine, oxysanguinarine, coptisine, behave as amino alcohols, since the process is carried out in an anhydrous medium. These alkaloids in the anhydrous state are known to be amino alcohols. Thus, for example, berberine, which in an anhydrous medium is termed berberinol, features the following structure:



In the course of interaction said alkaloids, behaving as amino alcohols, cause aminolysis of the thiophosphamide, that is, breaking of the ethylene imine rings which may be represented as



with the formation of an intraionic compound.

The products obtained by the reaction may be isolated by distilling-off the solvent from the reaction mixture, by washing-off the unreacted starting materials and recrystallization of the target product. When the target product is to be produced by reacting thiophosphamide with the mixture of alkaloids extracted from *Chelidonium majus*, the solvent is distilled off from the reaction mixture, the residue is washed to remove the unreacted starting materials, and the target product is isolated. The yield of the product is 32—97 wt. %.

The present invention will now be described, by way of illustration, in the following Examples.

Example 1

7.15 millimols of sanguinarine (M.P. 267°C) and 14.27 millimols of thiophosphamide are dissolved in 700 ml of benzene, and the mixture is refluxed in a flask during 2 hours. The resulting mixture is decolorized with activated charcoal and the solvent is distilled off. The dry residue is thoroughly washed with ether to remove the unreacted starting materials. 1.5 g of tri - (N - sanguinarinol - ethylamide) of thionophosphoric acid are obtained, which is a yellowish crystalline substance, well soluble in benzene, chloroform, dimethyl formamide and dichloroethane, sparingly soluble in water, soluble in 10% hydrochloric acid when heated, insoluble in methanol and ether. The yield is 50.8 wt. % of theory.

M.P. (from a mixture of chloroform and methanol) is 189—191°C. Absorption peak: 238, 338 and 407 nm.

$C_{66}H_{87}N_6O_{15}PS$. Calctd., in percent:
S, 2.59; N, 6.79; P, 2.50; C, 64.07; H, 4.64.

Found, in percent:
S, 2.70; 2.71; N, 6.82; 6.90; P, 2.45; 2.62; C, 63.90; 63.87; H, 4.60; 4.71.

Example 2

8.86 millimols of berberine (base) and 13.5 millimols of thiophosphamide are refluxed in a flask in 600 ml of anhydrous dioxane during 2 hours. The resulting mixture is decolorized with activated charcoal and the solvent is distilled off under a vacuum at 10 mm Hg. The dry residue is washed with ether and chloroform, 3.3 g of the N-berberinol - ethylamide of di - /ethyleneimido/ - thionophosphoric acid being thus obtained, which is a dark yellow crystalline substance, well soluble in hydrochloric acid under heating, and sparingly soluble in conventional organic solvents. The yield is 97 wt. % of theory. M.P. (from a mixture of benzene and dimethyl sulphoxide) is 135°C.

$C_{20}H_{21}N_4O_3PS$. Calctd. (in percent):
S, 5.91; N, 10.37; P, 10.33; C, 57.55; H, 5.76.

Found (in percent):
S, 5.90; 5.79; N, 10.52; 10.54; P, 10.41; 10.39; C, 57.40; H, 5.84.

Example 3

3.5 g of alkaloids obtained from an aqueous extract of *Chelidonium majus L* (average mole 331) and 3.8 g (20.1 millimols) of thiophosphamide are dissolved in 60 ml of chloroform and refluxed in a flask during 2 hours. The resulting product is decolorized with activated charcoal and the solvent is distilled off. The dry residue is thoroughly washed with ether to remove the unreacted starting materials, 1.45 g of the desired product are obtained, which is a light brown substance, easily soluble in chloroform, dimethyl sulphoxide and dimethyl formamide, sparingly soluble in dichloroethane, dioxane and methanol, and insoluble in water and ether. The yield is 34.5 ft. %.

For an approximate average molecular weight of 1120.

Calctd. (in percent): S, 2.86; N, 7.50.

Found (in percent): S, 2.82; N, 7.60.

Absorption peak: 284 nm.

Example 4

The process of interaction is carried out as described in Example 3, with benzene being used as the organic solvent.

The yield of the target product is 35 wt. %.

For an approximate average molecular weight of 1180.

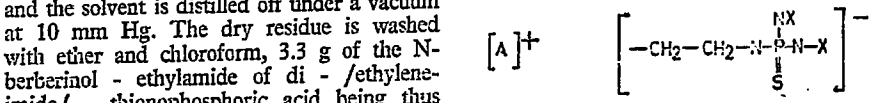
Calctd. (in percent): S, 2.72; N, 7.12.

Found (in percent): S, 2.64; 2.62; N, 7.30; 7.25.

Absorption peak: 271; 375 nm.

WHAT WE CLAIM IS:—

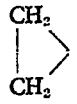
1. A compound of the general formula,



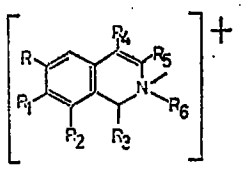
wherein X is the group

$$A—CH_2—CH_2—$$

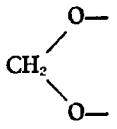
or the group



and A is the radical



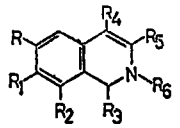
in which each of R, R_1 and R_2 is independently a hydrogen atom or a methoxy group or R and R_1 together or R_1 and R_2 together form a



group; R_3 is a hydrogen atom, a hydroxy group or a methyl group; each of R_4 and R_5 is independently a hydrogen atom or a dihydro-group and R_6 is a hydrogen atom or R_4 and R_5 together or R_5 and R_6 together form part of a fused substituted naphthalene ring.

2. A method of preparing a compound as claimed in claim 1 comprising reacting thio-

phosphamide with an isoquinoline alkaloid of the general formula



wherein R, R_1, R_2, R_3, R_4, R_5 and R_6 are as defined in claim 1, or with a mixture of the alkaloids of the said general formula in the medium of an organic solvent at the boiling point thereof.

3. A method as claimed in claim 2, wherein the organic solvent is benzene, chloroform, or dioxane.

4. A method as claimed in claim 3, wherein the alkaloid is berberine and the solvent is dioxane.

5. A method as claimed in claim 2 or 3, wherein the alkaloid is sanguinarine and the solvent is benzene.

6. A method as claimed in claim 2 or 3, wherein the alkaloid is a mixture of alkaloids extracted from *Chelidonium majus L.* and the solvent is benzene or chloroform.

7. A method of preparing a compound according to claim 1 according to any one of the Examples.

8. A medicinal preparation for treating malignant tumors comprising as an active principle a thiophosphamide derivative of an isoquinoline alkaloid as claimed in claim 1.

9. A medicinal preparation for treating malignant tumors, comprising as an active principle the N - berberinolethylamide of di-(ethyleneimido)thionophosphoric acid and a pharmaceutical carrier.

10. A medicinal preparation for treating malignant tumors, comprising as an active principle the tri - (N - sanguinarinol - ethyl-amide) of thionophosphoric acid and a pharmaceutical carrier.

11. A medicinal preparation as claimed in any one of claims 8 to 10, wherein the pharmaceutical carrier is a diluent of the following composition (in parts by weight): water, 1.5; polyethylene glycol (mol. wt. 400), 1.5; dimethyl sulphoxide, 2.

12. A medicinal preparation as claimed in any one of claims 8 to 11, containing the said active principle in an amount of 0.3 to 0.5 wt. %.

13. A medicinal preparation as claimed in any one of claims 8 to 10, wherein the pharmaceutical carrier is an emollient base for ointments.

14. A medicinal preparation as claimed in claim 13, wherein the emollient base is medicinal petroleum jelly and anhydrous lanoline and a diluent of the following composition (in parts by weight): water, 1.5; polyethylene glycol (mol. wt. 400), 1.5; dimethyl sulphoxide, 2.

15. A medicinal preparation as claimed in claim 13 or 14, comprising the said active principle in an amount of from 0.3 to 0.5 wt. %.

16. A medicinal preparation as claimed in any one of claims 8 to 10, wherein the pharmaceutical carrier is a pharmaceutical base for suppositories.

17. A medicinal preparation as claimed in claim 16, wherein the pharmaceutical base for suppositories is cacao oil and anhydrous lanoline and a diluent of the following composition (in parts by weight): water, 1.5; polyethylene glycol (mol. wt. 400), 1.5; dimethyl sulphoxide, 2.

18. A medicinal preparation as claimed in claim 16 or 17, comprising the said active principle in an amount of from 0.03 to 0.05 wt. %.

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